Pediatric abdominal injury

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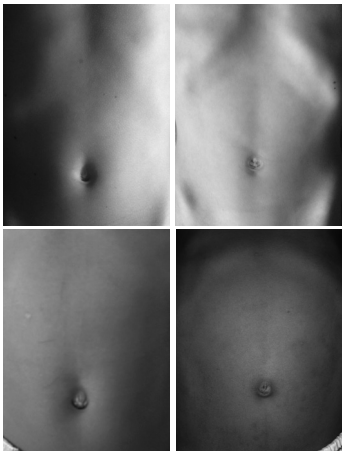
Chapter 6

Correlates and kinetics of L-FABP in multi-trauma patients: a potential novel marker for abdominal injury and injury severity



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ABSTRACT

Introduction

Blunt abdominal traumas present a diagnostic challenge. A specific marker for abdominal injury would be a valuable diagnostic asset. Liver Fatty Acid Binding Protein (L-FABP) is present in liver, kidney, pancreas and the intestines. Serum levels of L-FABP may thus be a marker for severity of injury and/or of intra-abdominal injury

Aim of this study was to establish kinetics of L-FABP plasma levels in multi-trauma patients. Secondary aim was to gain insight into the relation of L-FABP levels with the severity of injury and presence of intra-abdominal injury.

Patients/Methods

Prospective study including all consecutive adult patients admitted to the shock room with suspicion of multitrauma. Blood samples for L-FABP ELISA were obtained upon arrival (T0), and subsequently three (T3), six (T6), nine, 12 and 24 hours after trauma. L-FABP levels were correlated with clinical data, e.g. shock index, emergency trauma score (EMTRAS), Abbreviated Injury Score (AIS), Injury Severity Score (ISS), length of (ICU) stay and mortality.

Results

In 56 patients (46M, 10F, median age 46 years, median ISS of 17) median L-FABP plasma concentration was elevated on admission and decreased to normal levels within 9 hours. Half life time was just over three hours. Initial L-FABP levels correlate with ISS, abdominal AIS, EMTRAS, shock index, ICU stay and mortality. The five abdominally injured patients had higher median L-FABP levels at T0, T3 and T6.

Conclusion

Based on these results L-FABP can be seen as marker for injury severity, possibly specifically for presence of abdominal injury.

INTRODUCTION

Despite diagnostic and therapeutic advances, managing patients with blunt abdominal trauma still present a clinical challenge. The mechanism of injury, physical examination, hemodynamic status and several blood/serum analyses together with medical imaging help the attending physician to form his judgment. Extensive diagnostic work-up of the patients with potential abdominal injury often is time consuming and expensive. In addition, imaging techniques have limitations and are frequently operator or interpreter dependent. Unnecessary imaging may even be harmful to patients due to radiation exposure or the use of intravenous contrast. A specific serum marker for abdominal injury would be a valuable addition to the diagnostic workup. It could save valuable time, and reduce costs and unwarranted medical examinations.

Non operative management (NOM) of abdominal injuries is the treatment that is preferred over operative treatment. However, certain multi-trauma patients do need an operation or radiological intervention when deterioration occurs.[1] A sensitive indicator for such deterioration would aid in the observation of the abdominally injured patient.

Fatty acid binding proteins (FABP) are small proteins (12-15 kDa) that are present in various organs, including those of the abdomen and are involved in the intra cellular fat metabolism. Nine different isoforms are known in the human body. For the trauma surgeon probably the most relevant FABP's are I-FABP (intestinal) and L-FABP (liver). While I-FABP is mainly present in the enterocytes of the small bowel, L-FABP is present in the liver, the kidney, the pancreas and the small and large bowel. Tissue damage, due to injury and hypo perfusion of the splanchnic region as result of hemorrhagic shock, induces cell disruption. This leads to the release of FABP's into the systemic circulation. And as a result of their small molecular mass, FABP's might be subsequently rapidly excreted by the kidney. Due to its wide expression in the internal organs, L-FABP might be a sensitive marker for abdominal injury.

Several small studies suggest that I and L FABP may be a valuable adjunct to the diagnosis of abdominal injury, with higher FABP levels upon admission to the emergency room indicating the presence of intra-abdominal injury.[2-4] Aim of the present study is to establish the kinetics of L-FABP in multi-trauma patients. Secondary aim is to gain insight into the relation of L-FABP levels with the presence of intra-abdominal injury and the severity of injury

PATIENTS AND METHODS

Setting

The University Medical Centre Groningen is a Level I trauma centre covering the North of the Netherlands. Annually, some 250 multi-trauma patients are admitted to the Emergency Room, of which an estimated 10 to 20% will have intra-abdominal injury that may require surgical or radiological intervention.

Patients

To ensure a representative trauma population, all adult patients (≥18 years of age) admitted to the Emergency Room of the UMCG were eligible when serious injury was suspected, regardless of the type or extent of injury. The suspicion of serious injury was defined by the mobilisation of the UMCG A-trauma team, consisting of, among others, a trauma surgeon, an anesthesiologist, a radiologist and a neurologist. Upon admission, all patients were treated according to the Advanced Trauma Life Support (ATLS) guidelines. All patients therefore underwent X-rays of cervical spine, chest and pelvis. Ultrasonography of the abdomen was also performed in all cases. Computed Tomography (CT) scans were performed as deemed necessary by the attending trauma team. Indications for abdominal CT were the presence of free fluid or parenchymal injury on ultrasound or extensive concomitant injuries to head, chest and pelvis. The presence of intra-abdominal injury was defined by the presence of characteristic signs on ultrasound or CT scan or by intra-operative findings. Only injury to the intra-abdominal organs or hollow visceral injury was considered to be intra-abdominal injury, while fractures of the bony structures of the pelvis were not. The group was subsequently divided into patients with and without abdominal injury. Multivariate analysis was performed to establish the discriminating ability of L-FABP in identifying abdominal injury.

All consecutive multi-trauma patients admitted in May and July 2010 were evaluated. This prospective trial (NTR2211) that aims at evaluating the use of Fatty Acid Binding Proteins in the diagnosis of abdominal trauma and the development of complications such as multiple organ failure was approved by the Medical Ethical Committee of the University Medical Centre Groningen. Informed consent was obtained 48 hours after trauma from either patient or patient proxy, using the procedure of informed consent as described in previous studies.[5,6] If patients were to be discharged before this timeframe, informed consent was obtained before discharge. When informed consent was not obtained, samples were destroyed.

To assess the presence of shock, the Shock Index was computed.[7] We also calculated the Emergency Trauma Score (EMTRAS), based on base excess, Glasgow Coma Scale and prothrombin time as determined within 30 minutes of arrival to the ED.8 After discharge from the hospital, the abdominal Abbreviated Injury Score (AIS) and the Injury Severity

Score (ISS) were computed by a trained physician assistant according to the AAST 1998 updated revised organ injury scale.[9,10] Age, sex, Length Of Hospital Stay (LOHS), Intensive Care Unit Stay (ICUS) and in hospital mortality were also registered.

Blood Processing

To determine the kinetics of L-FABP, blood samples were obtained upon arrival in the emergency room (T0), and subsequently three (T3), six (T6), nine (T9), 12 (T12) and 24 (T24) hours after trauma. Thereafter daily blood samples were taken until surgery or until discharge, whatever came first. Specimens were collected in pre-chilled ethylenediaminetetraacetic acid (EDTA) vacuum tubes (Vacutainer®). All samples were instantly processed and centrifuged at 1500g at 4ºC for 15 minutes. Afterwards the supernatant was transferred to fresh polypropylene tubes and stored at -80 ºC until batch sample analysis.
Blinded specimens were used for duplicate measurement of L-FABP levels. A commercially available Enzyme Linked Immunosorbent Assay (ELISA) kit (HK 404, Hycult Biotechnology, Uden, the Netherlands) was used according to the manufacturers' instructions. The standardized undiluted detection limits were 100-25000 pg/ml. Samples were diluted until the detectable window was reached. Plasma of healthy individuals contains approximately 12000 pg/ml L-FABP. Three healthy subjects without a medical history served as controls.

Statistics

Continuous variables were compared using Student's t-test or Mann Whitney U-test as appropriate. Categorical data were compared using Chi-square or Fisher Exact test. For non-parametric correlations Spearman's test was used.
A p-value of < 0.05 was considered statistically significant. The Statistical Software Package (SPSS 16.0) was used for all analyses.

RESULTS

Patients

Between May and July 2010 there were 62 eligible patients. Denial of informed consent occurred in 1 patient and 5 patients were not enrolled for various reasons (see table 1). Patient characteristics of those not enrolled did not differ from the study group. (Data not shown)
There were 46 males and 10 females included in the study. Their median age was 46 yrs (18-82). Five patients sustained one or more abdominal injuries: liver (4), spleen (2), and duodenum (1). The median shock index of all patients was 0.79 (0.33-1.83), the median EMTRAS was 3.5 (0-9) and the median ISS was 17 (1-75). The median abdominal AIS was 0 (0-5). The median LOHS was 7 days (1-72) and ICU stay was 1 day (0-65) Mortality was

Table 1: Reasons for exclusion of study

	N
No obtained informed consent	1
Dead on arrival	1
Invalid/lost or no samples taken	3
Age under 18 after initial inclusion	1
Total	6

Table 2: Characteristics of the abdominally versus non abdominally injured patients

	Whole group	Abdominally injured	Non abdominally injured	p-value
N	56	5	51	0.35
Age*	46 (18-82)	48 (38-82)	45 (18-79)	
Sex	46 M 10 F	4M 1 F	42M 9F	0.35
ISS *	17 (1-75)	49 (17-59)	16 (1-75)	0.01
AIS abdomen*	0 (0-5)	4 (2-5)	0 (0-2)	0.00
EMTRAS*	3.5 (0-9)	4 (2-6)	3 (0-9)	0.597
Shock index*	0.71 (0.33-1.83)	0.86 (0.43-1.75)	0.69 (0.33-1.83)	0.19
LOHS*	7 (1-72)	11 (1-29)	6 (1-72)	0.17
ICUS*	1 (0-65)	2 (1-11)	1 (0-65)	0.085
Mortality	25%	40%	24%	0.42

Values are *median (range). ISS, Injury Severity Score. AIS, Abbreviated Injury Score. EMTRAS, Emergency Trauma Score. LOHS, Length of hospital stay in days. ICUS, Intensive Care Unit Stay in days.

25%. These characteristics, also divided in those who were abdominally injured versus those who were not, are depicted in table 2.

L-FABP kinetics

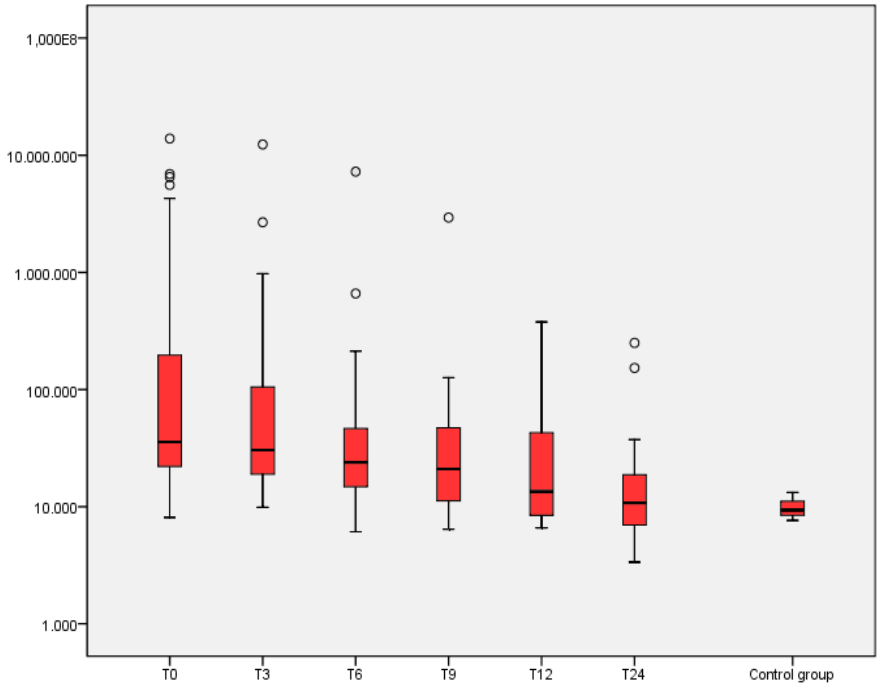
Blood for the T0 sample (arrival in Emergency Room) was obtained after a median of 54 minutes post-injury (range 44-150). Median L-FABP upon admission to the Emergency Room was 35660 pg/ml (8086-13,882,309). The median L-FABP concentration decreased sharply to normal levels within 9 hours as depicted in figure 1. The median half-life time of the decreasing L-FABP levels in the first few hours was 3 hours and twenty minutes.

Correlation of L-FABP with clinical parameters

There is a significant correlation between L-FABP levels upon admission and ISS, abdominal AIS, EMTRAS, Shock Index, ICU stay and mortality. Age, sex and LOHS did not correlate with L-FABP levels on admission. (Table 3) Also, levels of L-FABP were significantly higher in patients with a shock index above 0.7 (p=0.026).

The abdominally injured group had higher median L-FABP levels at T0, T3 and T6 compared to the non-abdominally injured group (p=0.06) (Figure 2).

Figure 1: Box plots representing L-FABP levels of all patients at the 6 different time moments. Y-axis in logarithmic scale



The seven box plots correspond with the first six samples (T0-T24) and the group of healthy controls. The margins of the box are the 25th and 75th percentile; the middle band depicts the median. Samples with L-FABP value above 1.5 times the interquartile distance are denoted by a circle.

Figure 2: Median plasma levels of L-FABP in patients with and without abdominal injury in time.

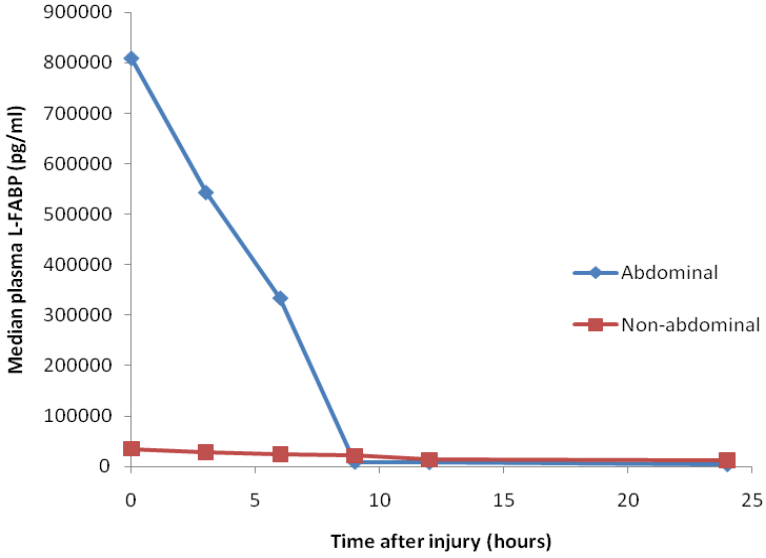


Table 3: Correlation between T0 L-FABP levels and clinical parameters.

T0	Correlation coefficient	p-value
Age	0.31	0.88
Sex	0,03	0.98
ISS	0.40	0.00*
AIS	0.34	0.01*
EMTRAS	0.49	0.00*
Shock index	0,51	0.00*
LOHS	0.09	0.52
ICU	0,32	0.02*
Mortality	0.27	0.04*

*Statistically significant

There results of our blood samples are depicted in figure 1. Several patients, who did not sustain abdominal injury, developed high levels of L-FABP and some levels stayed persistently high during the first days. Further analyses of these specific data indicate that the patients with high L-FABP who did not sustain intra-abdominal injury all were hemodynamically unstable neurotrauma patients who, in the ICU, required vasopressors to maintain an acceptable hemodynamic profile. Some patients with extensive abdominal and concomitant injuries persisted to produce high levels of L-FABP's through the first days. These patients were also sedated and admitted to the ICU where vasopressors and intravenous fluids were administered to treat hemodynamic instability.

DISCUSSION

Identification of patients with significant intra-abdominal injury is important to improve outcome. The reliability of radiological methods such as FAST (Focused Assessment with Sonography for Trauma) is variable and operator dependent, therefore the computed tomography (CT) is considered the gold standard for the diagnosis of intra-abdominal injuries. However, there is poor correlation between the findings on CT and outcome of (conservative) management of parenchymal injuries.[1] Likewise, grading of injury (as described by the AAST) using CT has never been validated. The only study trying to do so (in children) demonstrated only a moderate inter and intra observer agreement.[11]
In many hospitals CT is relatively time consuming due to transport to and from the CT scan. During transport and the scan itself, monitoring the patient is also more difficult. Overreliance may even result in poor outcome in specific cases.[12] Finally, the risk of cancer induction due to the large dose of radiation involved in CT scanning is becoming more

important, especially in the paediatric population.[13] Therefore it would be of the utmost importance to identify novel markers for abdominal injury.

This paper describes the kinetics of plasma L-FABP levels in a consecutive cohort of adult trauma patients. To our knowledge, this is the first study describing consecutive L-FABP levels in multitrauma patients. The present study demonstrates that there is an increase in L-FABP levels shortly after injury in multitrauma patients. L-FABP levels return to normal within several hours. In trauma patients, median L-FABP half-life time was just over three hours. Confirming the hypothesis that injury triggers L-FABP release, there is a significant increase of L-FABP levels in patients with a shock index > 0.7. There is a significant correlation between L-FABP levels upon admission with ISS, abdominal AIS, EMTRAS, Shock Index, ICU stay and mortality. There are some remarkably high levels in our study, specifically in the first measurements. These patients all suffered from severe brain injury, indicating that not only abdominal injury but also systemic injury elevates levels of L-FABP significantly. Further studies are needed to verify this hypothesis.

Although these results represent a relative small study, there is a large difference between patients with and patients without intra-abdominal injury, bordering on a statistically significant difference (p=0.06). This suggests that L-FABP might be a useful adjunct as an early indicator for intra-abdominal injury.

Another novel finding is the high level of L-FABP's in hemodynamically unstable patients with neurotrauma. We can only speculate that these comatose patients somehow constantly produce L-FABP possibly due to the hypoperfusion of the splanchnic area, which is associated with hypovolemic shock or possibly with the use of vasopressors such as nor epinephrine. Another possible explanation might be that the systemic inflammatory response causes the constant release of L-FABP in the systemic circulation. Finally, the release of L-FABP from neuronal tissue can not be ruled out, although the literature suggests that L-FABP is not present in brain or nerve tissue.[14,15] This goes beyond the scope of the present paper, yet needs further investigation.

Based on these preliminary results L-FABP can be seen as early marker for severity of injury and possibly specifically for the presence of abdominal injury. L-FABP levels rise quickly after the injury to rapidly decrease afterward. A larger number of patients will be necessary to determine the diagnostic accuracy of L-FABP for the presence of intra-abdominal injury.

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Part 3
Intra-abdominal injury in children